

## CLAIMS

1. A cell culture substrate having at least one area for culturing a cell on a substrate,  
5 characterized in that the culturing area comprises an area for holding a biologically active substance having a biological activity to the cell and an area for immobilizing a biologically active substance having a biological activity to the cell.
- 10 2. The cell culture substrate according to claim 1, wherein a plurality of biologically active substances are held or immobilized in at least either of the holding area and the immobilizing area in each culture area.
- 15 3. The cell culture substrate according to claim 1, wherein the biologically active substance in the holding area is held in such a manner that it is released in a culture liquid when coming in contact with the culture liquid.
- 20 4. The cell culture substrate according to claim 1, wherein at least either of the holding area and the immobilizing area in the culture area is provided in plural units.
- 25 5. The cell culture substrate according to claim 1, wherein the holding area and the immobilizing area include areas between which the kind of the biologically active substance or the

combination of plural biologically active substances is different, and one or more combinations are included.

6. The cell culture substrate according to  
5 claim 1, wherein the holding areas and the immobilizing areas include at least a combination of the areas different in a density of the biologically active substance.

7. The cell culture substrate according to  
10 claim 1, wherein the culture area is formed in a recess formed on a surface of the substrate.

8. The cell culture substrate according to claim 1, wherein the culture area is surrounded by a wall-shaped structure.

15 9. The cell culture substrate according to claim 1, wherein at least either of the holding area and the immobilizing area includes an area in which a biologically active substance is held or immobilized across a supporting layer provided on a surface of  
20 the substrate.

10. The cell culture substrate according to claim 1, wherein the holding area is provided at a predetermined height from a lower end of the culture area.

25 11. The cell culture substrate according to claim 10, wherein a culture area includes two or more holding areas provided in positions different in

distances from a lower end of the culture area.

12. The cell culture substrate according to claim 1, characterized in that the biologically active substance can be control-released or a  
5 biologically active substance having a control-release property can be liberated from the holding area.

13. The cell culture substrate according to claim 1, wherein the culture area is provided in a  
10 recess, of which at least one of walls is inclined from a bottom portion to an upper portion, and an aperture of the recess has an area wider than a bottom area of the recess.

14. A method for producing a cell culture  
15 substrate according to any one of claims 1-13, characterized in that liquid discharge means is utilized for providing a biologically active substance to at least one of the holding area and the immobilizing area.

20 15. The method according to claim 14, wherein the liquid discharge means is discharge means by a thermal ink jet method.

16. The method according to claim 14, wherein the liquid discharge means is discharge means by a  
25 piezo ink jet method.

17. The method according to claim 14, further comprising a step of carrying out fixation of the

biologically active substance by applying an immobilizing energy from the exterior.

18. A method for screening a cell culture condition utilizing a cell culture substrate  
5 according to any of claims 1-13, the method comprising the steps of:

filling the culture area with a culture liquid and culturing cells in a state where a biologically active substance immobilized in an immobilizing area  
10 of the culture area is in contact with the culture liquid; and

contacting the culture liquid with the holding area thereby liberating a biologically active substance present in the holding area into the  
15 culture liquid.

19. The method according to claim 18, further comprising the step of replenishing the culture liquid with a substance necessary for screening a cell culture condition.

20. The method according to claim 18, further comprising the step of observing a shape change of cells.

21. The method according to claim 20, wherein cells are stained for evaluation.

22. The method according to claim 18, further comprising the step of carrying out a quantitative determination of a substance synthesized in the cells.

23. The method according to claim 18, further comprising the step of carrying out a quantitative determination of a substance incorporated in the cells.

5           24. The method according to claim 22 or 23, further comprising a step of carrying out a quantitative determination of the substance by at least one of a radiation dose measurement, a fluorescence amount measurement, a light emission  
10 amount measurement and an optical absorbance measurement.